16 Factors Affecting Commercial Success: Case Studies in Cotton, Turf and Citrus

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16.1. Introduction

Commercialization of entomopathogenic nematodes has experienced highs and lows. Successes include control of the Diaprepes root weevil, *Diaprepes abbreviatus*, in citrus (Duncan *et al.*, 1996; Grewal and Georgis, 1998), the black vine weevil, *Otiorhynchus sulcatus*, in cranberries (Georgis *et al.*, 1991), billbugs, *Sphenophorus* spp. in turf (Smith, 1994), and fungus gnats (Diptera : Sciaridae) in mushrooms and greenhouse plants

(Gouge and Hague, 1995; Grewal and Georgis, 1998). But for every success there have been numerous failures. In many cases, success was not achieved despite the pests having shown promising susceptibility in laboratory or field trials, e.g. Colorado potato beetle, *Leptinotarsa decemlineata* in vegetables and corn (Wright *et al.*, 1987), the corn earworm, *Helicoverpa zea* in corn (Cabanillas and Raulston, 1996) and cockroaches in urban and industrial environments (Appel *et al.*, 1993). Entomopathogenic nematodes are pathogenic to over 200 insect hosts (Poinar, 1979; Klein, 1990), yet nematodes have only been successfully marketed for a small fraction of these insects. The objective of this chapter is to examine the factors that influence the success or failure of biocontrol programmes with entomopathogenic nematodes. Case studies of insects in three different cropping systems serve as a basis for our analysis. The systems were chosen to illustrate an example in which nematodes failed (cotton), have been somewhat successful (turf), and have succeeded (citrus).

16.2. Cotton

Cotton is the most important natural textile fibre in the world. The cotton plant is a perennial species but it is cultivated as an annual to reduce pest populations from year to year. Insect pests can cause significant reductions in cotton yield and quality (Schwartz, 1983). Economic thresholds depend upon a variety of factors including the cotton growth stage damaged and the predicted value of cotton at harvest. Several cotton pests are susceptible to entomopathogenic nematodes including bollworm, *H. zea*, fall armyworm, *Spodoptera frugiperda*, beet armyworm, *Spodoptera exigua*, cabbage looper, *Trichoplusia ni*, tobacco budworm, *Heliothis virescens*, and the pink bollworm, *Pectinophora gossypiella* (Raulston *et al.*, 1992; Henneberry *et al.*, 1995a; Gouge *et al.*, 1999). This section will focus on pink bollworm because most research on control of cotton pests with entomopathogenic nematodes has been conducted on this organism.

Pink bollworm is an introduced pest, first appearing in the US in 1917 in infested seed brought from Mexico (Spears, 1968). Today pink bollworm is a key pest of cotton grown in the western states, and a constant threat to the cotton regions of California's San Joaquin Valley. Currently pink bollworm is established in Arizona, California, New Mexico, parts of Oklahoma, Florida and Western Texas, but also occurs in Arkansas, Louisiana and Missouri.

Pink bollworm overwinter as diapausing larvae, then pupate and emerge in spring and early summer (Bariola and Henneberry, 1980). Feeding by larvae reduces lint and seed production, and reduces lint quality. Boll infestation also exacerbates problems caused by plant pathogens such as *Aspergillus flavus*, by offering organisms an avenue of infection. From 1966 to 1980, annual losses in California's Imperial Valley averaged 26% of the crop value; as a result cotton hectarage has declined significantly in this area (Naranjo *et al.*, 1995). Current control consists of chemical sprays targeting adults during the main boll developmental period and using transgenic varieties with insecticidal properties.

16.2.1. Development of nematodes for pink bollworm management

In the early 1990s, pink bollworm larvae were found to be highly susceptible to *Steinernema carpocapsae* and *S. riobrave* (Lindegren *et al.*, 1992, 1993a, 1994). Particular interest was generated in *S. riobrave* because it was isolated from a lepidopteran host in

an arid region of the Lower Rio Grande Valley in Texas (Cabanillas *et al.*, 1994). *Steinernema riobrave* has a better host (*Galleria mellonella*) searching efficiency compared with *S. carpocapsae* (Lindegren *et al.*, 1993a) and is more tolerant to the high temperatures (Henneberry *et al.*, 1996a) prevalent in western USA cotton agroecosystems. Small plot trials indicated potential for entomopathogenic nematodes to be a useful tool in pink bollworm management (Lindegren *et al.*, 1993b; Henneberry *et al.*, 1995a,b, 1996b). For example, treatment of Arizona fields showed that *S. riobrave* persisted in large numbers for 19 days and were recoverable up to 75 days following treatment (Gouge *et al.*, 1996). The number of infested bolls was reduced and yield increased by 19% compared with untreated fields. Similar results were obtained with *S. riobrave* and *S. carpocapsae* in Texas cotton fields (Gouge *et al.*, 1997). *S. carpocapsae* and *S. riobrave* are the only species tested under field conditions.

16.2.2. Factors affecting efficacy

16.2.2.1. Phenology

The field efficiency of biological control agents depends upon coordinating application of the agent with the susceptible insect stages. Laboratory and field studies have indicated two stages of the pink bollworm lifestage that are susceptible to entomopathogenic nematodes in soil: pre-pupae and diapausing larvae (Henneberry *et al.*, 1995a; Gouge *et al.*, 1997). Pupae are not susceptible to infection by steinernematid nematodes (Henneberry *et al.*, 1995a).

16.2.2.2. Environmental factors

Irrigation during nematode application and continued moderate soil moisture are essential for nematode movement, persistence and virulence (Georgis and Gaugler, 1991). Henneberry *et al.* (1996b) noted increased pink bollworm mortality in plots treated with *S. riobrave* after successive field irrigations. As nematode applications require irrigation, only areas growing cotton under irrigated conditions could hope to use the nematodes effectively (T.J. Henneberry, Arizona, 2000, personal communication). Except for a small hectarage in eastern New Mexico, all western cotton is irrigated. However, across the USA only 37% of cotton is irrigated, which would be of consequence if pink bollworm establishes in central and eastern areas.

Temperature limits the virulence of steinernematids by its influence on nematode activity, bacterial symbiont, or both (Kaya 1990; Grewal *et al.*, 1994). Subterranean temperatures in cotton fields rarely rise higher than 28°C, but surface temperatures may exceed 50°C. *Steinernema riobrave* infects pink bollworm at temperatures up to 36°C with maximum infection occurring at 28.5°C, whereas *S. carpocapsae* and *H. bacteriophora* have optimum infection at 25°C (Gouge *et al.*, 1999). Other nematode species are capable of infecting insects at high temperatures, including *S. glaseri*, *S. anomoli* (Grewal *et al.*, 1994), and *H. indica* (Shapiro and McCoy, 2000a), but have not been assessed as pink bollworm control agents.

16.2.2.3. Application methods and timing

Entomopathogenic nematodes have been applied to control pink bollworm through irrigation systems (Lindegren *et al.*, 1992; Forlow Jech and Henneberry, 1997), sub-surface application with a shanking or disc system (K.A. Smith, Arizona, 2000,

personal communication), and diverse spray equipment including aerial application (Gouge *et al.*, 1997). The methods have not been compared experimentally, but application directly on to irrigated soil or application via irrigation water would logically cause less nematode mortality due to desiccation and ultraviolet radiation compared with other methods.

Application of nematodes during pre-plant irrigation to control diapausing larvae is undoubtedly the most convenient strategy, utilizing the advantages of cooler soil temperature and uniform distribution to soil within fields unimpeded by plants. However, pink bollworm moths emerging from diapause are highly mobile (Flint and Merkle, 1981) and unless this strategy is adopted on an area-wide basis, protection of localized areas will be short-lived. After cotton plants have become established and flowering begins, movement of the moths becomes increasingly restricted (Flint and Merkle, 1981) and applications of nematodes to smaller areas is more beneficial.

Greatest pink bollworm mortality has been achieved using application rates of 2.5×10^9 infective juveniles per ha during early season applications (92.5 and 100% for *S. carpocapsae* and *S. riobrave* respectively, Henneberry *et al.*, 1996b), and 3.25×10^9 infective juveniles ha^{-1} during mid-season (100% furrow base mortality, Gouge *et al.*, 1996). The efficiency of multiple applications, with standard or reduced rates, has yet to be explored.

16.2.3. Current status and analysis

Although entomopathogenic nematodes are highly efficacious in controlling pink bollworm, they are not a viable management strategy because they cannot compete against the current management tactics. Chemical pesticides have been relied upon heavily for pink bollworm management, but current trends make transgenic cotton the most popular option. Toxin genes from *Bacillus thuringiensis* (BT) bacteria have been incorporated directly into the cotton plant creating transgenic varieties with insecticidal properties. In 2000, approximately 70% of cotton grown in Arizona was BT-cotton. BT-cotton is far more economical than nematodes, and unlike nematodes, will provide control of several major cotton pests simultaneously, including pink bollworm, bollworm and tobacco budworm. Cotton growers are charged an US$87 fee per ha for transgenic cotton-seed, whereas nematode costs, using a single application rate of 2.5×10^9 ha^{-1}, are US$312.50 ha^{-1} for *S. riobrave*. Furthermore, the ecological advantage of entomopathogenic nematodes relative to chemical insecticides is essentially irrelevant since BT-cotton also appears to have minimal impact on non-targets and the environment. Finally, another advantage BT-cotton and chemical insecticides have over nematodes is ease of use. Small wonder that entomopathogenic nematodes are not used in cotton.

The future for entomopathogenic nematodes in pink bollworm management does not look bright. Transgenic cotton varieties have had a profound impact on the way pests are managed throughout the cotton belt. The major concern of BT-cotton has been widespread development of insect resistance to the BT toxins, but this has so far proven to be minimal, and new transgenic cotton varieties are under development which use gene stacking technology, i.e. multiple genes coding for different modes of action. The future of transgenic cotton appears stable for some time to come. Thus, pink bollworm appears to be a prime example of an insect that can be managed using

entomopathogenic nematodes but for practical reasons nematodes will remain unused in conventional cotton.

The only niche where entomopathogenic nematodes could play a role in cotton pest management is in the organic, coloured cotton market. Coloured, organic cotton values are approximately two to three times that of traditionally grown cotton making use of nematodes more feasible. However, interest in growing organic coloured cotton fell in the mid 1990s, and unless this trend is reversed, it is likely the potential for using nematodes in cotton will disappear completely.

16.3. Turf

Turfgrass comprises a variety of grass species grown as a permanent or semi-permanent managed ground cover under a range of management systems (e.g. lawns, parks, cemeteries, sod farms, golf courses, athletic fields). Between systems there are large variations in value, input, demands, damage thresholds and, consequently, tolerances for pests. Damage thresholds are generally low; therefore numerous insects are considered pests (Potter, 1998; Vittum *et al.*, 1999). Among the important insect pests several are amenable to control by entomopathogenic nematodes. This section will concentrate on those pest species that have received the most attention as targets for nematodes, i.e. white grubs (Coleoptera: Scarabaeidae), mole crickets (*Scapteriscus* spp.), billbugs (*Sphenophorus* spp.) and the black cutworm (*Agrotis ipsilon*). Other pests that have been controlled with nematodes include annual bluegrass weevil (*Listronotus maculicollis*), cutworms, armyworms, sod webworms and European crane fly (*Tipula paludosa*).

16.3.1. White grubs

White grubs, the root-feeding larvae of scarab beetles, are serious pests of turfgrass throughout the world. Most important species have an annual life cycle with adults emerging in summer. The eggs are laid in the soil below the turf. By late summer most larvae have developed into the third and final instar. After overwintering the larvae resume feeding in spring until pupation in early summer. The extensive feeding activity of the larger larvae can kill large areas of grass especially under warm dry conditions. In addition, vertebrate predators can tear up the turf to feed on the grubs even at relatively low larval densities.

16.3.1.1. Development of entomopathogenic nematodes for control of white grubs

No other turfgrass pest has been studied as extensively as a target for nematodes as white grubs (Klein, 1990, 1993). As soil insects, white grubs are predisposed to nematode attack and many nematode species and strains have been isolated from white grubs (Peters, 1996). However, as a result of their coevolution with soil pathogens, white grubs have developed various defensive mechanisms that have conferred to them varying degrees of nematode resistance among grub species.

The isolation of *S. glaseri* from Japanese beetle, *Popillia japonica*, larvae in New Jersey resulted in the first effort to use entomopathogenic nematodes for pest control (Glaser, 1932; Fleming, 1968). Even though a large-scale colonization programme failed due to lack of awareness of the nematode's symbiotic bacteria (Gaugler *et al.*, 1992), the effort built the base for further development of entomopathogenic nematodes as biological control agents.

Renewed efforts to develop nematodes for white grub control were triggered by the commercialization of entomopathogenic nematodes in the early 1980s. Generally *Heterorhabditis* spp. and *S. glaseri* were found to be more effective than *S. feltiae* and *S. carpocapsae* (Klein, 1990, 1993). Most field tests in the USA concentrated on *S. carpocapsae* and *H. bacteriophora* because these species were readily available. An analysis of 82 field trials conducted against *P. japonica* between 1984 and 1988 (Georgis and Gaugler, 1991) concluded that *H. bacteriophora* strains used under the right conditions were as effective as standard insecticides, whereas the most widely used species, *S. carpocapsae*, was ill-adapted for white grub control. The 1990s were characterized by more in-depth studies of factors affecting nematode efficacy against white grubs (see below), and advances in production technology. The development of liquid culture for *Heterorhabditis* spp. (see Chapter 14) increased production efficiency making the use of nematodes for white grub control more feasible.

16.3.1.2. Factors affecting efficacy

Nematode efficacy against white grubs is affected by various interacting biotic and abiotic factors. The thickness of thatch, an accumulation of organic matter between the soil and turfgrass foliage, is negatively related to nematode efficacy because thatch restricts nematode downward movement (Georgis and Gaugler, 1991; Zimmerman and Cranshaw, 1991). Nematodes, especially *H. bacteriophora*, become increasingly ineffective for white grub control as soil temperatures drop below 20°C (Georgis and Gaugler, 1991). Irrigation volume and frequency and soil moisture are positively related to efficacy (Shetlar *et al.*, 1988; Georgis and Gaugler, 1991). Nematodes are more effective in fine-textured soils because finer soils retain moisture better and restrict nematode movement to the upper soil layers where most of the white grubs can be found (Georgis and Gaugler, 1991).

As a result of their co-evolution with soil pathogens, white grubs have developed defence mechanisms including infrequent carbon dioxide output, sieve-plates over their spiracles, frequent defecation, defensive and evasive behaviours, a dense peritrophic membrane, and a strong immune response. To optimize nematode efficacy, it is crucial to identify nematode species that have adapted to white grub ecology and biology. Generally, highly mobile cruiser-type nematodes, e.g. *Heterorhabditis* spp. or *S. glaseri* are better adapted to infecting sessile subterranean insects such as white grubs than ambushers like *S. carpocapsae* (Gaugler *et al.*, 1997a; see Chapter 10). These differences in search strategy explain the superiority of *H. bacteriophora* and *S. glaseri* over *S. carpocapsae* in Japanese beetle control (Georgis and Gaugler, 1991; Georgis and Poinar, 1994). Differences have also been found in the interactions between the nematode–bacterium complex and the white grubs' immune system (Cui *et al.*, 1993; Wang *et al.*, 1995; see Chapter 4). While all nematode species elicit a strong immune response in *P. japonica* larvae, most *H. bacteriophora* are killed by melanotic encapsulation, but *S. glaseri* escape encapsulation.

Although direct comparisons are rare, it is apparent that nematode efficacy against white grubs varies with scarab and nematode species. For example, the Oriental beetle, *Exomala orientalis*, is less susceptible to *H. bacteriophora* than *P. japonica*, whereas the European chafer, *Rhizotrogus majalis*, or the Asiatic garden beetle, *Maladera castanea* are resistant to this nematode (A.M. Koppenhöfer, unpublished data). A recently isolated *Heterorhabditis* spp., however, was highly effective against the Asiatic garden

beetle but less effective against Oriental beetle and European chafer (R. Cowles, Connecticut Agricultural Experiment Station, 2000, personal communication). Larval stage also affects nematode susceptibility but the trend varies with scarab species (Deseö *et al.*, 1990; Fujiie *et al.*, 1993; Smits *et al.*, 1994). Differences in the expression of defensive mechanisms may contribute to differences in nematode susceptibility. Defensive and evasive behaviours are much stronger in *P. japonica* than in masked chafer, *Cyclocephala hirta* (Koppenhöfer *et al.*, 2000), yet the latter are less susceptible to nematodes suggesting differences in other defence mechanisms.

16.3.1.3. Current status and analysis

Despite considerable efforts in research and development, nematode use against white grubs is limited. The diversity of white grub pests, and their varying degree of susceptibility to different nematode species, has added to the difficulty. However, there has been some success. Two companies in Japan have recently started to market *S. glaseri* (SDS Biotech K.K.) and *S. kushidai* for white grub control on golf courses in Japan and a third company in Germany is marketing *H. bacteriophora* (E-Nema Gmbh) for the same market in Germany. In the USA, several small companies produce *Heterorhabditis* spp. for use against white grubs but the extremely high price (in the range of US\$1000 ha^{-1} or more) of these *in vivo* produced nematodes restricts their use to small area application such as in a homeowner setting.

Low economic thresholds and competition from chemical insecticides have played major roles in hindering wider nematode success. The control efficacy obtained under the right conditions against the Japanese beetle alone should have led to a quicker and more extensive acceptance of nematodes. But at a cost of upwards from US\$500 ha^{-1}, *in vitro* products containing *Heterorhabditis* spp. are more than four times as expensive as similarly effective organophosphate and carbamate insecticides. The phasing out of these 'harder' chemicals by regulatory agencies could have been a boon for nematode products, but the arrival of a new generation of 'low-impact' insecticides continues to impede commercialization of nematodes for white grub control. The cost of these new chemicals (e.g. imidacloprid and halofenozide) is around US\$250 ha^{-1}. Because their efficacy declines with advancing grub development, these chemicals must be applied on a preventative basis before white grub outbreaks can be identified, and thus involve the treatment of large turf areas that may need only partial or no treatment. Even though the preventative use makes these chemicals ultimately more expensive than the curative use of nematodes, they are extremely effective (close to 100% control) and relatively safe and therefore an attractive management option, especially where cost is not a major issue (e.g. many golf courses). Consequently, nematodes are presently used for white grub control only in countries where chemical alternatives are restricted (e.g. Germany, Japan) or in small commercial segments such as the homeowner market.

Despite potential for improving nematode utility in the future (e.g. through reduced production costs, more pathogenic nematode species and strains, and better understanding of white grub–nematode interactions), the success of nematodes as biopesticides for white grubs is likely to remain limited by competition from chemical insecticides. A more promising future for nematodes in white grub management may lie in developing alternative approaches to their use as biopesticides. For example, conservation and, even better, manipulation of the widespread

natural nematode populations in turfgrass could be used to buffer white grub outbreaks.

16.3.2. M le crickets

Mole crickets were introduced into Florida from South America around 1900 and have since become the most important turfgrass pest in the southeastern USA. Adults and nymphs cause damage by feeding on grass roots and shoots and through their extensive tunnelling activity. After egg-laying in spring the adults die off. During summer the crickets are in the nymphal stages until adults appear in late summer. Overwintering occurs primarily in the adult (tawny mole cricket, *S. vicinus*) or nymphal stage (southern mole cricket, *S. borellii*).

16.3.2.1. Development of entomopathogenic nematodes for control of mole crickets

Initial efforts showed that *S. carpocapsae* could provide some control of mole crickets in the field (average of 58% at 2.5×10^9 nematodes ha^{-1}) (Georgis and Poinar, 1994). However, exploration for natural enemies in South America led to the isolation of a new nematode species, *Steinernema scapterisci*, from *Scapteriscus* mole crickets in Uruguay and Argentina. The ensuing introduction of *S. scapterisci* into the USA for mole cricket control (Parkman and Smart, 1996) is the first successful use of an entomopathogenic nematode in classical biological control. After laboratory studies indicated excellent control potential and nontarget safety of *S. scapterisci*, the nematode was released on 1-ha plots in pastures in 1985 (Parkman *et al.*, 1993a) and on golf courses in 1989 (Parkman *et al.*, 1994). The nematode established successfully and mean cricket trap catches had declined by 98% within 3 years. The nematode was slowly spread from release sites by infected mole crickets (Parkman *et al.*, 1993b). Establishment was also achieved by applying *S. scapterisci*-infested cadavers and using electronic mating callers to attract mole crickets to the site of application (Parkman *et al.*, 1993a). These experiments demonstrated *S. scapterisci*'s ability to establish itself permanently or act as an inoculative agent, rather than an inundative one.

When *S. scapterisci* became commercially available, in 1993, its potential as an inoculative agent was enhanced. *S. scapterisci* provided the same control levels as standard insecticides (75% at 2.5×10^9 nematodes ha^{-1}) (Georgis and Poinar, 1994). The widespread use of *S. scapterisci* on golf courses, other turf areas, and pastures greatly accelerated the spread of *S. scapterisci*.

16.3.2.2. Factors affecting efficacy

Laboratory and field studies showed that the efficacy of *S. scapterisci* was affected by mole cricket species and developmental stage (Hudson and Nguyen, 1989a,b; Nguyen and Smart, 1991; Parkman and Frank, 1992). The short-winged mole cricket, *S. abbreviatus* was less susceptible than *S. vicinus* and *S. borellii* in laboratory studies. In addition, *S. borellii* was more susceptible than *S. vicinus* in field studies, probably because the greater activity arising out of its predatory behaviour increases it chances of contact with the ambusher *S. scapterisci*. Nymphal mole crickets were substantially less susceptible to infection than adults, and small nymphs were not affected by *S. scapterisci*. In addition to *S. scapterisci*, *S. riobrave* has been marketed for mole cricket control in turf (Grewal and Georgis, 1998). *S. riobrave* has shown the same efficacy for

mole cricket control and is also ineffective against mole crickets nymphs, but it does not recycle in infected mole crickets (K. Smith, University of Arizona, 2000, personal communication).

16.3.2.3. Current status and analysis

Perhaps the most significant aspect of the *S. scapterisci*-mole cricket system is the nematode's ability to recycle. It is likely that the nematode will become established throughout most of the mole crickets' area of distribution in the USA (Frank and Parkman, 1999). *S. scapterisci* is an ideal control agent for pastures and turfgrass areas that can tolerate some mole cricket damage. Because of the nematode's slow spread from inoculation sites, widespread use or an inoculation programme are necessary to accelerate its spread. This, however, has been hampered by the nematode's limited availability. There have been several periods when *S. scapterisci* has not been commercially available. Economics played a major role in the nematode's rocky development. At a cost of about US\$240 ha^{-1}, *S. scapterisci* was far more expensive than insecticides commonly used in pastures but comparable to some of the new insecticides used on turf. More importantly, the limited effect of *S. scapterisci* on mole cricket nymphs requires its application in spring or fall when adults are present, while control measures are typically necessary in summer against nymphs. In addition, the performance of *S. scapterisci* was variable in southeastern Florida where the less nematode-susceptible *S. abbreviatus* is abundant. Finally, lack of aggressive promotion and limited supplies contributed to poor acceptance in the market-place. In the last several years the role of nematodes in mole cricket control has dwindled to nothing (neither *S. riobrave* nor *S. scapterisci* are available for mole cricket control at this time). However, sparks of interest have been rekindled. Recently MicroBio, a company with a proven ability to mass produce entomopathogenic nematodes in liquid culture, obtained an exclusive license to produce and sell *S. scapterisci* (G. Gowling, MicroBio Cambridge, UK, 2000, personal communication). Time will tell if this company (or another) will enable nematodes to play a more prominent role in mole cricket control.

16.3.3. Billbugs

Billbugs, *Sphenophorus* spp., are important turfgrass pests throughout much of the USA and Japan. The younger larvae feed inside the stem and crown and older larvae feed externally on the below-ground parts of the plant. Seasonal life cycles vary depending on species and latitude. No detailed studies on billbug-nematode interaction have been published. Field tests in Ohio indicated that the bluegrass billbug, *Sphenophorus parvulus* can be controlled with *S. carpocapsae* (average 78%) or *H. bacteriophora* (average 74%) (Georgis and Poinar, 1994; Smith, 1994). In Japan, *S. carpocapsae* has been more effective for control of the hunting billbug, *S. venatus vestitus* than standard insecticides (average 84% versus 69% control), (Smith, 1994; Kinoshita and Yamanaka, 1998). Use of nematode products containing *S. carpocapsae* and *H. bacteriophora* against billbugs is limited in the USA, whereas *S. carpocapsae* is the primary means of billbug control on golf courses in Japan. The main reason for this difference is the availability of effective insecticides for billbug control in the USA and lack thereof in Japan. In addition, favourable environmental conditions (temperature and rainfall) and the adoption of 'nematode-friendly' application protocols, i.e. immediate watering after

spraying and generally very careful following of label instructions may have optimized nematode efficacy in Japan (K. Smith, University of Arizona, 2000, personal communication).

16.3.4. Black cutworm

The black cutworm is a perennial problem on the close-cut bentgrass of golf course greens and tees throughout the world. The larvae dig a burrow in the thatch or soil and emerge at night to chew down the grass blades and stems around the burrow. There are multiple generations per year. Georgis and Poinar (1994) reported that *H. bacteriophora* has not provided satisfactory control (average 62%), whereas *S. carpocapsae* is highly effective for black cutworm control (average 95%). Despite this high efficacy, nematodes are not widely used for black cutworm control. Economics does not play a significant role on these high-profile turf areas. Rather, damage thresholds on golf course tees and especially greens are so low that golf course superintendents will prefer to use chemical insecticides that provide even better and more consistent cutworm control than nematodes. This will continue until the attitude of their clientele changes.

16.4. Citrus

Citrus is a long-lived perennial evergreen that is grown in an orchard cropping system. Insect pests can cause substantial reductions in citrus yields and fruit quality (Browning, 1999). Economic thresholds depend on whether the pest causes direct or indirect damage, how severe the damage is, and whether the fruit is intended for fresh marketing or processing (into juice or other citrus products). A number of citrus pests have been tested for susceptibility to entomopathogenic nematodes including the citrus leafminer, *Phyllocnistis citrella* (Beattie *et al.*, 1995), the Mediterranean fruit fly, *Ceratitis capitata* (Lindegren *et al.*, 1990), the Caribbean fruit fly, *Anastrepha suspensa* (Beavers and Calkins, 1984) and the fuller rose beetle, *Asynonychus godmani* (Morse and Lindegren, 1996). By far, the greatest amount of research on entomopathogenic nematode control on citrus pests has been towards suppression of the weevils that threaten citrus root systems in Florida and the Caribbean (Duncan *et al.*, 1999; McCoy *et al.*, 2000a), primarily *Pachnaeus* spp. and *Diaprepes abbreviatus*. The remainder of this section and analysis will focus on this citrus root weevil complex emphasizing *D. abbreviatus*, which is the most serious insect pest of citrus in Florida (Duncan *et al.*, 1999).

Diaprepes abbreviatus was first reported in Florida in 1964 (Woodruff, 1964) and now infests more than 15% of Florida citrus (Duncan *et al.*, 1999). The life cycle is reviewed by McCoy (1999). Adult weevils emerge from soil throughout the year with a significant peak in the spring (March–June) and occasionally another peak between October and December (Stansly *et al.*, 1997). These adults feed and oviposit on foliage, and first instars drop to the ground where they burrow into soil and feed on roots. Economic damage is caused by larval feeding and is often exacerbated by the fungal disease *Phytophthora* spp., which can enter the roots at points of larval feeding (Duncan *et al.*, 1999). Although chemical insecticides can be applied (e.g. carbaryl) as an adulticide, or as a barrier treatment for neonates, the only recommended control for *D. abbreviatus* larvae that have established themselves in the orchard is application of entomopathogenic nematodes (Bullock *et al.*, 1999a).

16.4.1. Development of entomopathogenic nematodes for control of *D. abbreviatus*

Steinernema carpocapsae was the first nematode shown to be pathogenic to *D. abbreviatus* (Laumond *et al.*, 1979) and to be developed commercially for citrus root weevil control (Smith, 1994). Laboratory, greenhouse studies and field studies demonstrated some potential for *S. carpocapsae* to control *D. abbreviatus* and indicated higher virulence relative to *S. glaseri* and *S. feltiae* (Schroeder, 1987; Figueroa and Roman, 1990; Table 16.1). Reported mortality levels of *D. abbreviatus* in field studies, however, were not high (i.e. not > 70%), varied greatly, and often involved excessively high rates of application (Table 16.1).

The commercialization of entomopathogenic nematodes for citrus root weevil control changed course when a nematode that had been recently discovered, *S. riobrave*, was tested for efficacy towards *D. abbreviatus*. *Steinernema riobrave* caused greater *D. abbreviatus* mortality than *S. carpocapsae* in laboratory, greenhouse and field tests (Schroeder, 1994; Duncan *et al.*, 1996; Bullock *et al.*, 1999b). Several studies reported ≥ 90% suppression of *D. abbreviatus* in field studies with *S. riobrave* (Duncan and McCoy, 1996; Duncan *et al.*, 1996; Bullock *et al.*, 1999b). The encouraging research led to the commercial development of *S. riobrave* for control of *D. abbreviatus* replacing *S. carpocapsae* as the nematode of choice (Grewal and Georgis, 1998). In 1999, approximately 19,000 ha of citrus were treated with *S. riobrave* to control citrus root weevils (M. Dimock, Thermo Trilogy Corporation, Columbia, Maryland, 2000, personal communication). In addition to *S. riobrave*, *H. bacteriophora* and *H. indica* have been commercialized for use against *D. abbreviatus*, based on efficacy reported in laboratory or field studies (Shapiro *et al.*, 1999; Table 16.1).

Table 16.1. Field efficacy of *Steinernema* and *Heterorhabditis* nematodes against *Diaprepes* root weevil.

Nematode	Application rate (cm^{-2})	Percentage mortality	Reference
H. bacteriophora	127	78	Suggars Downing *et al.*, 1991
H. bacteriophora	255	63	Suggars Downing *et al.*, 1991
H. bacteriophora	637	63	Suggars Downing *et al.*, 1991
H. bacteriophora	100	62	Schroeder, 1992
H. bacteriophora	250	ns[a]	Duncan and McCoy, 1996
H. bacteriophora	175	54	Duncan *et al.*, 1996
H. bacteriophora	255	57	Duncan *et al.*, 1996
S. carpocapsae	250	65	Schroeder, 1987
S. carpocapsae	25	42	Schroeder, 1990
S. carpocapsae	100	50	Schroeder, 1992
S. carpocapsae	637	48	Suggars Downing *et al.*, 1991
S. carpocapsae	153	ns	Duncan *et al.*, 1996
S. carpocapsae	306	ns	Duncan *et al.*, 1996
S. glaseri	250	35	Schroeder, 1987
S. riobrave	250	77–90	Duncan and McCoy, 1996
S. riobrave	120	93	Duncan *et al.*, 1996

[a] ns, no suppression, i.e., mortality was not significantly different from the untreated control.

16.4.2. Factors affecting efficacy

Although entomopathogenic nematodes have been successfully commercialized to control *D. abbreviatus* in Florida citrus, levels of suppression have varied greatly (Table 16.1). The remainder of this section will focus on factors influencing the success (or failure) of entomopathogenic nematode applications for citrus root weevil control.

When applying entomopathogenic nematodes to control any insect pest, the choice of nematode species is perhaps the most critical aspect to achieving an efficacious application. (Georgis and Gaugler, 1991; Gaugler, 1999). Control of *D. abbreviatus* with nematodes may be considered a case in point. If the superiority of *S. riobrave* to *S. carpocapsae* had not been discovered it is likely that the market for nematodes in Florida citrus would have eventually failed. Thus far, *S. riobrave* has proven to be the most effective nematode for *D. abbreviatus* control. In laboratory studies, *S. riobrave* was found to be more virulent to seventh or eighth instar *D. abbreviatus* than eight other nematode species (*H. bacteriophora, H. indica, H. marelatus, H. megidis, H. zealandica, S. carpocapsae, S. feltiae, S. glaseri*) and 17 strains (Shapiro and McCoy, 2000a). In another laboratory study, however, *H. indica* was found to be more virulent than *S. riobrave* against younger instar *D. abbreviatus*, i.e. fourth–fifth instar (Shapiro *et al.*, 1999). In a greenhouse study, using potted citrus, *S. riobrave* caused higher *D. abbreviatus* mortality than *H. bacteriophora* (against seventh and 11th instars) and *H. indica* (against seventh instars) (Shapiro and McCoy, 2000b). In field studies, *S. riobrave* is the only nematode reported to have caused 90% or more mortality of *D. abbreviatus* (Bullock *et al.*, 1999b; Table 16.1).

In addition to virulence, nematode persistence could be an important factor in determining which nematode to apply (Shields *et al.*, 1999). Stability and favourable soil conditions (moisture, aeration, texture) make Florida citrus groves amenable to entomopathogenic nematode recycling and persistence (see Kaya, 1990). Indeed, endemic nematode populations exist (Beavers *et al.*, 1983) and can provide significant *D. abbreviatus* suppression (e.g. 7–42%) (McCoy *et al.*, 2000b). However, inundative applications of nematodes (i.e. *H. bacteriophora, H. indica, S. carpocapsae* and *S. riobrave*) to citrus have resulted in poor persistence, reaching pre-treatment levels within 2 weeks post-application (Duncan *et al.*, 1996; McCoy *et al.*, 2000b). Discovery of other species that are both virulent and persistent would be beneficial.

Formulation and culture method (*in vivo* versus *in vitro*) may also affect entomopathogenic nematode efficacy (Gaugler and Georgis, 1991; Baur *et al.*, 1997). With regard to control of *D. abbreviatus* with *S. riobrave*, however, no significant effects of culture method or formulation (liquid versus granular) were detected (Duncan and McCoy, 1996; Shapiro and McCoy, 2000c). In contrast to virulence, the viability of granular formulated *S. riobrave* has been reported to be variable (McCoy *et al.*, 2000b; Shapiro and McCoy, 2000c). Variations in viability may not be detrimental to efficacy because the manufacturer of the granular *S. riobrave* (Thermo Trilogy Corporation) packs an excess of nematodes to each unit to ensure that at least the labelled amount of viable nematodes are available during the shelf-life of the product (M. Dimock, Thermo Trilogy Corporation, Columbia, Maryland, 2000, personal communication). Additionally, low viability does not appear to affect the virulence of nematodes that remain alive during the product's shelf life (Shapiro and McCoy, 2000c). Nonetheless, variation in viability can be a considerable hindrance to grower acceptance of the

product; low nematode viability in several batches of *S. riobrave* (detected by the producer, distributors, or researchers) has resulted in product recalls and subsequent lowering of consumer confidence. *In vivo* produced *Heterorhabditis* spp. formulated in sponge or as paste and *in vitro S. riobrave* in liquid formulation tend to have high viabilities (e.g. > 90% and > 75%, respectively; McCoy *et al.*, 2000b).

Mortality of *D. abbreviatus* is positively related to rate of nematode application (McCoy *et al.*, 2000b). In general, a minimum of 25 infective juveniles per cm^2 is required to achieve adequate pest suppression (Georgis and Hague, 1991; Gaugler *et al.*, 2000). In citrus, however, high levels of *D. abbreviatus* control (i.e. > 85%) have only been achieved with application rates exceeding 100 infective juveniles per cm^2 (Table 16.1). Industry recommended application rates for *D. abbreviatus* control are substantially lower, i.e. approximately ten infective juveniles per cm^2 for *Heterorhabditis indica* (Grubstake™, Integrated BioControl Systems Incorporated, Lawrenceburg, IN) and 22 per cm^2 for *S. riobrave* (Biovector®, 355 Thermo Trilogy Corporation, Columbia, Maryland).

16.4.3. Current status and analysis

Factors that have contributed to the commercialization of entomopathogenic nematodes in citrus for control of *D. abbreviatus* and other root weevils are biological, ecological, and economical in nature. From a biological standpoint, successful commercialization has been achieved because a proper match between the nematode and target-pest was made. From an ecological standpoint, Florida citrus groves contain many benefits for applying entomopathogenic nematodes. The soils in these groves have a high sand content (Shapiro *et al.*, 2000) facilitating nematode mobility (Georgis and Poinar, 1983; Barbercheck and Kaya, 1991) and oxygen availability for nematode survival (Kung *et al.*, 1990). Because *D. abbreviatus* occurs primarily under the tree canopy, nematodes only need to be applied within the drip line (Duncan *et al.*, 1999; McCoy *et al.*, 2000a) where shade protects them from harmful ultraviolet radiation (Gaugler and Boush, 1978). Additionally, most Florida citrus groves are irrigated, thus moisture, which is necessary for nematode survival, (Kaya, 1990), is provided.

The importance of economic factors leading to successful commercialization of nematodes for *D. abbreviatus* control cannot be underestimated. To begin with, *D. abbreviatus* is a key pest, and thus grower demand for control is high. Furthermore, in management of *D. abbreviatus* larvae, nematodes face little or no competition from other control agents (Bullock *et al.*, 1999a; McCoy, 1999). Inexpensive chemical insecticides (e.g. chlorinated hydrocarbons) have been eliminated due to regulatory pressures and, thus far, the newer chemicals that have been tested for *D. abbreviatus* larval control have not been able to compete with nematodes in price or efficacy. Indeed, perhaps the most important success-contributing factor is nematode cost. Because *D. abbreviatus* only occur under the canopy there is no need to apply nematodes between rows or between trees. Therefore the number of nematodes (and the cost) required to treat 1 ha of citrus may be three to ten times lower than crops that require broadcast applications to the entire soil surface (e.g. cotton and turf). The low cost of nematode application (e.g. as low as US\$62 ha^{-1}) combined with the high value of citrus (US\$6000 gross ha^{-1} for oranges, Muraro *et al.*, 1999), adds greatly to the success of entomopathogenic nematodes in Florida citrus.

Entomopathogenic nematodes are likely to continue to be an integral part of pest management in Florida citrus. The role of nematodes in controlling *D. abbreviatus* will be enhanced by continued research and improved quality control. Factors that affect efficacy such as those described previously must continue to be studied. Additionally, the role endemic entomopathogenic nematodes play in the citrus ecosystem has only begun to be examined. How non-indigenous nematodes that are applied interact with the endemic nematode populations, as well as with other soil biota (e.g. ants, McCoy *et al.*, 2000b), must be explored. The influence of soil characteristics (Shapiro *et al.*, 2000) and timing and methods of nematode application must also be investigated further (Duncan *et al.*, 1999; McCoy, 1999; Shapiro *et al.*, 1999). Finally, perhaps the most important issue yet to be resolved will be to determine the minimum application rate necessary to ensure efficacy and protect trees from economic damage.

16.5. Conclusions

Based on the case studies and other literature we have examined (e.g. Klein, 1990; Georgis *et al.*, 1991; Grewal and Georgis, 1998; Table 16.2), we can make certain generalizations. We conclude that two basic elements are necessary for nematodes to be successful: a suitable nematode for the target pest and favourable economics. For example, nematodes associated with control of *D. abbreviatus*, *O. sulcatus*, and sciarid pests are all highly suited to their hosts, are applied in relatively high value commodities, and face little or no competition from other control measures. Where entomopathogenic nematodes have not succeeded, the causes are generally due to a poor match of nematode and host, or poor economic conditions. For example, *S. riobrave* proved to be highly efficacious toward pink bollworm, but in cotton, nematodes could not compete on an economic level with other control strategies. Other insects in row crops fall into the same category, e.g. *H. zea* (Cabanillas and Raulston, 1996), and *Diabrotica* spp. (Jackson, 1996) in maize. In other instances a suitable match of nematode to target pest could not be found, e.g. wireworms (Coleoptera: Elateridae) (Eidt and Thurston, 1995) and imported fire ants *Solenopsis invicta* (Drees *et al.*, 1992), in which case strategies to employ entomopathogenic nematodes are futile. Other factors that may affect the success of commercial ventures with entomopathogenic nematodes include efficacy of pest suppression relative to other available control tactics, and reliable provision of a nematode product of high quality.

Proper match of the nematode to the host entails virulence, host finding, and ecological factors. If a nematode does not possess a high level of virulence toward the target pest there is little hope of success. In rare cases persistence may compensate for moderate virulence (Shields *et al.*, 1999). Matching the appropriate nematode host-seeking strategy with the pest is also essential (Lewis *et al.*, 1992; Gaugler, 1999). Nematodes that have an ambush strategy are most suitable for controlling mobile insects near the soil surface (e.g. *S. carpocapsae*), whereas nematodes with more of a cruiser strategy (e.g. *H. bacteriophora*) are most suitable for suppressing less mobile insects below the soil surface (Lewis *et al.*, 1992). Ecological factors such as relative ability to withstand desiccation or temperature tolerance are also important in choosing the best-adapted nematode for a particular pest.

Historically, poor host suitability has been the most common cause of failure in entomopathogenic nematode applications (Gaugler, 1999). The list of pests that

Table 16.2. An analysis of host suitability for entomopathogenic nematodes against various insect pests.[a]

Pest	Nematode species	Host suitability[b] (% suppression)	Number of references in analysis
Black vine weevil (*Otiorhynchus sulcatus*)	*Heterorhabditis bacteriophora*	Good (71)	7
	Steinernema feltiae	Good (75)	3
	S. carpocapsae	Fair (58)	5
Colorado potato beetle (*Leptinotarsa decemlineata*)	*S. carpocapsae*	Fair (57)	3
Corn rootworms (*Diabrotica* spp.)	*S. carpocapsae*	Good (61)	7
Japanese beetle (*Popillia japonica*)	*H. bacteriophora*	Excellent (80)	7
	S. carpocapsae	Fair (47)	6
	S. glaseri	Good (63)	3
White grubs (*Phyllophaga* spp.)	*H. bacteriophora*	Good (72)	3
Chafers (*Cyclocephala* spp.)	*H. bacteriophora*	Fair (59)	3
Sciaridae (*Lycoriella* spp. and *Bradysia* spp.)	*S. feltiae*	Excellent (89)	5
Leafminer (*Liromyza trifolii*)	*S. carpocapsae*	Good (66)[c]	3
Black cutworm (*Agrotis ipsilon*)	*S. carpocapsae*	Excellent (86)	5
Diamondback moth (*Plutella xylostella*)	*S. carpocapsae*	Fair (56)	3
Corn earworm (*Helicoverpa zea*)	*S. riobrave*	Excellent (90)[d]	4
Borers (*Synanthedon* spp.)	*S. feltiae*	Excellent (86)	4
Spodoptera spp.	*S. carpocapsae*	Poor (27)[e]	3
Imported fire ant (*Solenopsis invicta*)	*S. carpocapsae*	Poor (25)	3

[a] Only pests (by genus or species) with at least three refereed publications on field efficacy for each nematode species were included, and only if the rate of nematode application was not excessive (i.e. not > 125 nematodes cm^{-2}).
[b] Host suitability ratings: Excellent, Good, Fair, and Poor are based on suppression levels of 80–100, 60–79, 40–59, and < 40%, respectively. The suppression levels were calculated by averaging results from field trials in the associated references.
[c] Indoor applications in high humidity.
[d] Soil applications.
[e] Above ground applications.

commercial nematode-producing companies advocate as targets has frequently been inflated (Gaugler *et al.*, 2000). Mortality caused under laboratory conditions has often been inappropriately extrapolated to field efficacy (Georgis *et al.*, 1991). Perhaps the

greatest example of exaggeration in host range has been with *S. carpocapsae* which, for many years, was practically seen as a 'cure all' (Gaugler, 1999).

The definitive test of host suitability is efficacy under field conditions. We suggest a minimum of three solid field trials to establish host suitability. An analysis of nematode host suitability (based on field efficacy) for some of the most extensively studied insect pests is illustrated in Table 16.2. The table only includes host-nematode combinations with at least three refereed publications on field efficacy. The best matches tend to be for nematodes that have high virulence toward hosts in a protected environment, e.g. Japanese beetle, fungus gnats, borers, etc. Nematode applications to environments exposed to ultraviolet radiation or desiccation, e.g. *Spodoptera* spp., are prone to failure. Behavioural aspects of the target host may also be a factor, e.g. fire ants' ability to relocate after nematode treatment (Drees *et al.*, 1992).

If economic factors are not favourable even a strategy involving the most suitable match of nematode to target pest is doomed to failure. Economic factors include the grower's perceived need to control the pest, the relative cost of nematodes compared with other management options, the value of the commodity (e.g. per ha), and the overall importance of the commodity in the agricultural market. Exemption of entomopathogenic nematodes from the Environmental Protection Agency pesticide registration has clearly benefited commercialization efforts. Niche markets tend to be amenable to entomopathogenic nematode use not only because the crop value is high, but also because the commodity occupies a small enough segment of the agricultural market for would-be competitors to shy away from registration costs and seek alternatives. On the other hand, major row crops, such as maize, cotton, soybeans and wheat are, and likely to always be, unreachable for entomopathogenic nematode marketing because the crop value is low, and the market segment is huge.

A number of measures can be taken to improve the success of entomopathogenic nematodes. In the past 10 years new opportunities in pest control have arisen due to the discovery of several new entomopathogenic nematode species, including *S. riobrave* (Cabanillas *et al.*, 1994) against the *Diaprepes* root weevil (Table 16.1) and plant parasitic nematodes (Grewal *et al.*, 1997), *H. indica* (Poinar *et al.*, 1992) for the *Diaprepes* root weevil (Shapiro *et al.*, 1999), *H. marelatus* (Liu and Berry, 1996) for *Otiorhynchus* spp. (Berry *et al.*, 1997) and *S. scapterisci* (Nguyen and Smart, 1990) for mole crickets (Parkman *et al.*, 1994). Continued discovery of novel entomopathogenic nematodes, or novel uses for them, is certain to lead to new and improved pest control. Use of nematodes may also be expanded by increasing host suitability through genetic improvement (Gaugler, 1987; Gaugler *et al.*, 1997b; Shapiro *et al.*, 1997) or better formulation. Improvements in production technology, distribution, and application will be a key to reducing nematode costs and insuring quality. In this vein, Gaugler (1997) proposed local level cooperatives to produce nematodes cheaply and effectively for on-site use. Application of nematodes in infected hosts instead of aqueous suspension may also be a potential approach (Shapiro and Lewis, 1999), which could reduce costs of *in vivo* production because several labour-intensive steps would be avoided.

The continued pressure for regulating harmful chemical pesticides, particularly from the Food Quality Protection Act, will favour development of entomopathogenic nematodes and other biocontrol agents. Conversely, discovery of novel 'environmentally friendly' chemicals such as imidacloprid and halofenozide will continue to inhibit wider nematode use. The future of entomopathogenic nematodes in

existing markets is likely to remain stable for some time, and several new markets with favourable host suitability and economics will arise. Target pests that may become successful markets for nematodes in the near future include adult pecan weevils, *Curculio caryae* (Shapiro-Ilan, 2001) in pecans, and plant parasitic nematodes (Grewal *et al.*, 1997) in turf and vegetables. Furthermore, a new frontier is being opened by using entomopathogenic nematodes, and more so, their symbiotic bacteria or associated metabolites, as anti-microbial agents in pesticide and pharmaceutical applications (Li and Webster, 1997). However, major leaps in technology will be necessary before nematodes become successful biopesticides in markets presenting the most challenging barriers such as low value row crops and outdoor above-ground applications.

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